B-5559

APPLICATION FOR RESEARCH GRANT FROM NATIONAL SCIENCE FOUNDATION

INSTITUTION: The Rockefeller Institute, New York 21, N.Y.

PRINCIPAL INVESTIGATOR: Rollin D. Hotchkiss

TITLE OF PROPOSED RESEARCH: Physical Chemistry of Deoxyribonucleates having Genetic Transforming Activity

PROPOSED STARTING DATE: July 1, 1960

PERIOD FOR WHICH SUPPORT REQUESTED: Five years

ABSTRACT OF PROPOSED RESEARCH:

The physical and chemical parameters which define the maximally active state of bacterial deoxyribonucleate will be investigated by the techniques of genetic transformation. Selective inactivation by degrading and denaturing agents will be studied, in conjunction with selective enrichment by purification of individual genetically active deoxyribonucleate fractions. Both will be correlated with molecular structure as determined by physical methods.

Hotchkiss

Rollin D. Hotchkiss, Ph.D., principal investigator
Member and Professor, Laboratory of Genetics

Frank L. Horsfall, Jr., M.D.
Vice-president

DESCRIPTION OF PROPOSED RESEARCH:

Background---The ability to use chemically defined preparations of deoxyribonucleate (DNA) as the active entity in certain bacterial transformations makes possible a kind of experimentation that has thus far had to remain indirect in other genetic systems, even though most of them are thought to depend upon DNA. It is proposed to intensify the efforts begun some years ago directed toward the elucidation of the functional significance of various elements of the DNA chemical structure. We have shown that various kinds of denaturation lead to inactivation of biologically active genetic markers: heat, acid, strong base, and deoxyribonuclease (and perhaps phospho-diesterase) destroy activity. Zamenhof, and Lerman, Goodgal and others have meanwhile approached somewhat more systematically further aspects of the effects of these agents and radiation. Several laboratories have undertaken chromatographic fractionation of DNA, including ourselves, with promising, though not yet convincing results. Doty and his group have greatly clarified the concepts and methodology for the physical examination of the macromolecular structure of dissolved DNA. With Dr. Muriel Roger we have recently shown with these methods that pneumococcal DNA differs significantly from the classical calf thymus DNA in being a more extended, less flexible rodlike molecule, probably of somewhat lower molecular weight. Our laboratory has furthermore spent much effort to develop suitable genetic markers and outline the criteria for quantitative genetic studies with DNA.

Proposal ---The time seems appropriate for an intensified examination of the light-scattering, sedimentation and viscosimetric parameters of intact, fractionated and selectively modified DNA, in correlation with biological activity. Methods have been developed here for marginal denaturation by heat, acid, radiophosphorus (P^{32}) decay, and enzymatic digestion; and other workers have provided the background for modification by such agencies as low ionic strength, nitrous acid, or ionizing radiation. All of these have bearing on theoretical conceptions about the molecular structure of DNA. The structural studies will be correlated with several levels of biological activity: 1) ability of genetic markers to be imprinted or incorporated, 2) ability of specific sub-genic portions of a marker to recombine and so to be imprinted or incorporated, 3) ability of isotopic phosphorus or carbon atoms labeling the DNA nucleotide structure to be incorporated, 4) the effect of radioactive decay of P^{32} upon the activity of already incorporated DNA-P^{32}, and 5) the effect of even heterologous or degraded DNA can be assessed by measuring its competitive effect upon the ability of cells to fix or express genetic or isotopic properties carried by homologous active DNA. Methods for determining all of these have reached some degree of quantitative precision as they have been developed in this laboratory for several mutant markers during the past few years.

Thus, the general aim will be to define which of these biological functions are dependent upon which specific features of the structure of intact or modified deoxyribonucleate molecules.

The system used will involve principally the pneumococcus, various mutant strains interacting with the DNA from other strains and in some cases DNA from other organisms. Concurrent other work of this laboratory will be directed toward analysis of the changes, qualitative and quantitative, in protein synthesis induced by these DNA preparations, and the mode of integration of these DNA factors into the genome of the cells being transformed.

LITERATURE CITATIONS: About thirty papers on nucleic acids and transformation have appeared from this laboratory in the last ten years. General ones and ones bearing specifically upon the present project include:

Hotchkiss, R.D., The genetic chemistry of the pneumococcal transformations, Harvey Lectures, 41, 124-144 (1953-54).

Hotchkiss, R.D. and J. Marmur, Double marker transformations as evidence of linked factors in deoxyribonucleate transforming agents, Proc. Natl. Acad. Sci., 40, 55-60 (1954).

Hotchkiss, R.D., Criteria for quantitative genetic transformation of bacteria, in A Symposium on the Chemical Basis of Heredity, McElroy and Glass, eds., Johns Hopkins Press, Baltimore, 1957, 321-335.

Fox, M. S. and R. D. Hotchkiss, Initiation of bacterial transformation, Nature, 179, 1322-1325 (1957).

Fox, M. S., Deoxyribonucleic acid incorporation by transformed bacteria, Biochim. Biophys. Acta., 26, 83-85 (1957).

Roger, Muriel and R. D. Hotchkiss, On the molecular weight and shape of transforming deoxyribonucleate from pneumococcus, (to be submitted shortly to J. Amer. Chem. Soc.).

Hotchkiss, R. D. and A. H. Evans, Analysis of the complex sulfonamide resistance locus of pneumococcus, Cold Spring Harbor Sympos. Quant. Biol., 23, 85-97 (1958).

PERSONNEL:

Rollin D. Hotchkiss, principal investigator, born 1911, Conn.; B.S. chemistry, Yale 1932; Ph.D. organic chemistry, Yale, 1935, Loomis Fellow, 1934-35; Rockef. Foundation Fellow, Carlsberg Laboratory, Copenhagen, 1937-38. At Rockefeller Institute, New York, 1935-37, 1938 to present, progressively fellow, asst., associate, associate member; member and professor since 1955. Membership and participation in various societies, and committees. Fields of interest: pyrimidine chemistry 1933-35; immunochemistry 1935-37; protein chemistry, 1937-46; antibiotics, peptides, mode of action of drugs, 1940-46; transformation, genetics, nucleic acid chemistry, 1946-1960.

Muriel Roger (Mrs. Charles O. Beckmann), will be chiefly occupied on this project and supported by it with exception of salary from The Rockefeller Institute; born 1922, New York; C.C.N.Y. evenings 1938-47, chemistry; B.S. cum laude, Columbia 1950, chemistry; Ph.D. chemistry, Columbia 1952; Secretarial positions, 1938-45; at Columbia Chemistry Dept.: secretary-technician 1945-47; research assistant, 1947-50; research fellow, 1950-54; research scientist, A.E.C., 1954-55; at Rockefeller Institute, U.S.P.H.S. Fellow, 1955-56; research associate, 1956 to present; publications and thesis, starch and polymer chemistry, unpublished work on transformation.

Maurice S. Fox, partly occupied on this project, supported by The Rockefeller Institute totally as to salary; born 1924, New York; Queens College, 1941-43, 1946-47; B.S. meteorology, U. Chicago, 1944; M.S., Ph.D., physical chemistry, 1947-51. At Univ. Chicago, asst., Chemist, research associate

PERSONNEL (continued)

and instructor, 1947 thru 1951; in microbiol. genetics 1952-53; at Rockefeller Institute, research associate, 1953-56; assistant professor, 1956 ----. Nuffield Scholar, 1957; Lalor Foundation Fellow, 1959. Publications in radiochemistry, mutation, and transformation, partly included above.

Research assistant, to be appointed.

FACILITIES: A modern bacteriological and biochemical laboratory of three large rooms and accessory space such as preparation rooms and coldroom, with somewhat more space in prospect. Equipment includes usual temperature controlled baths and rooms, spectrophotometers, centrifuges of continuous flow, refrigerated and Spinco types, light-scattering photometer, isotope counting equipment, etc. The personnel of this laboratory group includes the principal investigator and three experienced investigators in genetics or physical chemistry and a variable group of younger associates and students. The larger environment of this Institute includes the group around Dr. Edward L. Tatum and many others with related interests.

BUDGET PROPOSAL:

Salaries AND Employee Benefits	Research assistant		$ 7500	
	Secretary, part time		500	$ 8000
Permanent Equipment		Microbalance	1300	
		Flow counter	500	1800
	(in future years, a fluorimeter and a titrimeter, centrifuge equipment)			
Supplies and Expendables		Chemicals and glassware	1800	
		Isotopic chemicals	500	
		Bacteriological media	700	3000
Travel expenses	Annual meetings, especially of specialists working on transformation		700	700
Publication costs	Reprints and preprints		250	250
		SUBTOTAL		$ 13,750
Indirect costs, 20% of subtotal				2,750
		TOTAL FOR FIRST YEAR		$ 16,500

Estimates, 2nd through 5th years
4 x $16,500 $ 66,000

OTHER SUPPORT: This aspect of our work is not supported by any source outside the Rockefeller Institute, and no application for such support is in prospect. Besides postdoctoral fellows, our group is supported by only one grant, U.S.P.H.S. Grant E-3170 covering another part of our work (metabolism of drug resistant mutants, five years, $21,282 per year).

Rollin D. Hotchkiss